

Investigating the impact of polarization on foamy macrophage induction by amiodarone *in vitro*

BACKGROUND

Foamy macrophage responses are often observed in histological lung slices of rats from pre-clinical studies *in vivo*, which are typically characterised by a high vacuolated appearance and larger cell size^{1,2,3}. The mechanism of induction of the foamy alveolar macrophage phenotype is not well known and it has not been explained if these observations are triggered by macrophage polarization.

AIM

Investigate changes in polarization and lipid content of the human foamy macrophages.

METHODOLOGY

1. CELL CULTURE

Human monocyte cells (U937) were seeded into 96-well plates at a density of 3×10^4 cells/well. U937 cells were differentiated to a macrophage phenotype using 4nM PMA in complete culture media for 96h followed by a 24h rest period in complete culture media.

2. INDUCTION AND ACTIVATION OF MACROPHAGE RESPONSES

Differentiated cells were activated to M1 macrophages by IFN γ (20ng/mL) and LPS (100ng/mL), or M2 macrophages by IL-4 (25ng/mL). Following 24 hours activation, cells were incubated with 10 μ M amiodarone or 50 μ M etoposide in cell culture medium to induce foamy phenotype.

3. M1/M2 FLOW CYTOMETRY DISCRIMINATION

Flow cytometry (Guava 8HT, Millipore, UK) was used to assess an expression of following polarization markers: M0: CD11b (surface marker, BD Bioscience, UK) M1: CXCL10 (intracellular marker, BD Bioscience, UK) and M2: CD206 (surface marker, BD Bioscience, UK).

4. ASSESSMENT OF LIPID CONTENT

For detection of phospholipids cells were incubated with HCS LipidTOX Green Phospholipidosis (Invitrogen, UK) fluorescent dye and analysed immediately after staining on Guava 8HT flow cytometer (Millipore, UK).

RESULTS

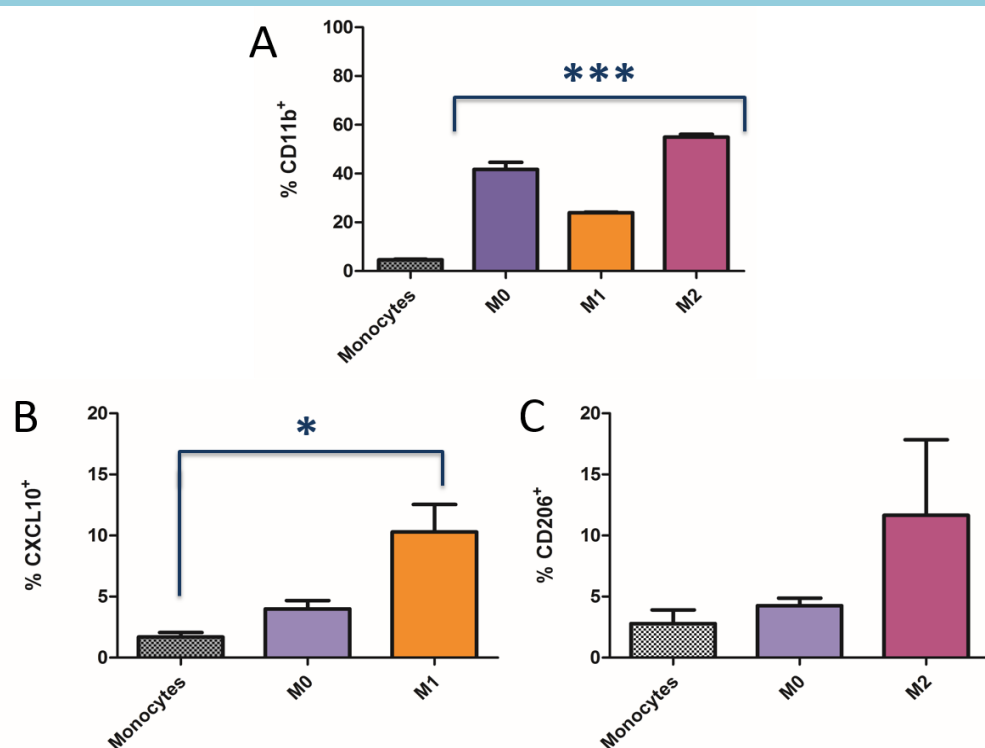


Figure 1: Assessment of U937 differentiation and M1 or M2 activation. U937 cells were differentiated to M0 alveolar-like macrophages, followed by 24 h activation to M1 and M2 state. Multicolour flow cytometry was used to discriminate expression of macrophage polarization markers. Results are presented as percentage of cells expressing: CD11b – M0 marker (A) CXCL10 – M1 marker (B) and CD206 – M2 marker (C). Data is shown as the mean \pm standard deviation of two separate experiments, each counting 5000 cells. *** $p < 0.001$, * $p < 0.05$.

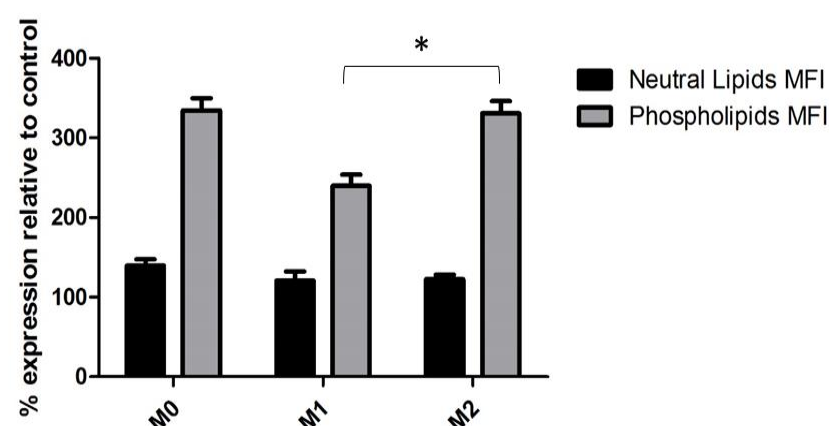


Figure 2: Assessment of neutral lipids and phospholipids accumulation in U937 cells treated with amiodarone. U937 cells were differentiated to M0 alveolar-like macrophages, activated to M1 and M2 state and treated for 24h with 10 μ M amiodarone. Neutral lipids and phospholipids content was analysed using flow cytometry. Results are presented as a percent expression of neutral lipids (black bars) or phospholipids (grey bars) relative to untreated control. Data is shown as the mean \pm standard deviation of three separate experiments, each counting 5000 cells. * $p < 0.05$.

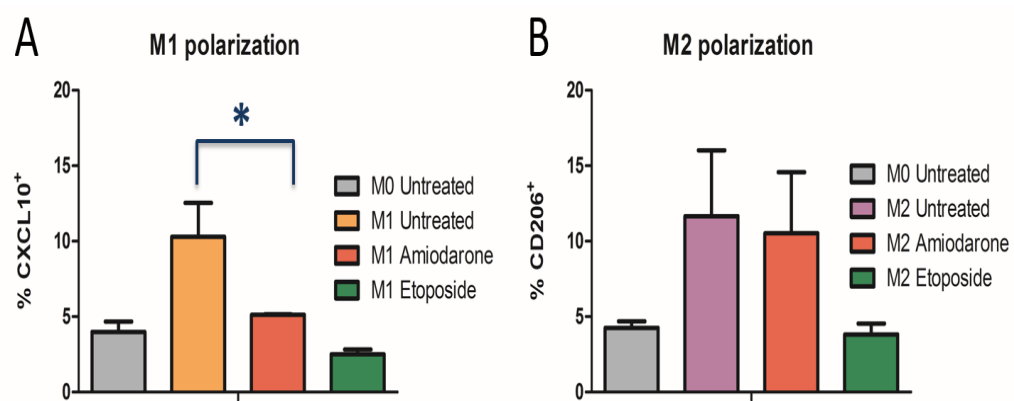


Figure 3: Effect of foamy macrophage inducers on expression of macrophage M1 - (A) and M2 - polarization (B) markers. U937 cells were differentiated to M0 alveolar-like macrophages, activated to M1 and M2 state and treated for 48h with 10 μ M amiodarone or 50 μ M etoposide. Multicolour flow cytometry was used to discriminate macrophage polarization markers expression. Results are presented as percentage of cells expressing: CXCL10 – M1 marker (A) and CD206 – M2 marker (B). Data is shown as the mean \pm standard deviation of two separate experiments, each counting 5000 cells. * $p < 0.05$

CONCLUSION

Amiodarone in 10 μ M concentration decreased the CXCL10 production reversing the cell M1-polarization, which resulted in lower phospholipids accumulation. The results suggest that amiodarone toxicity may be mediated by pathways involved in M1 macrophage activation.

Improved understanding of macrophage subtype responses to inhaled stimuli is necessary for further progress in this area, and to fully characterise the foamy macrophage response to elucidate its role in airway pathophysiology.

REFERENCES

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- 3) Hoffman E, et al. *Mol Pharmaceutics*. 2015;12(8):2675-2687